The Journal of Trace Elements in Experimental Medicine 9:215–229 (1996)

Evidence for the Nutritional Essentiality of Boron

Forrest H. Nielsen*

United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota

Since 1981, circumstantial evidence has been accumulating which suggests that boron is an essential nutrient for higher animals including humans; that is, a dietary deprivation of boron consistently results in changed biological functions that could be construed as detrimental and are preventable or reversible by an intake of physiological amounts of boron. Nonetheless, boron is not generally recognized as essential or nutritionally important for humans, probably because a specific biochemical function for boron has not been elucidated, or as demonstrated for plants, it has not been shown necessary to complete the life cycle. However, findings from human and animal experiments show that boron is a dynamic trace element that can affect the metabolism or utilization of numerous substances involved in life processes including calcium, copper, magnesium, nitrogen, glucose, triglyceride, reactive oxygen, and estrogen. Through these effects, boron can affect the function or composition of several body systems including blood, brain, and skeleton. Two hypotheses have appeared to account for these multiple effects. One hypothesis is that boron is a negative regulator that influences a number of metabolic pathways by competitively inhibiting some key enzyme reactions. The other hypothesis is that boron has a function involved in cell membrane function, stability, or structure such that it influences the response to hormone action, transmembrane signalling, or transmembrane movement of regulatory cations or anions. Regardless of the fact that the function of boron remains undefined, the findings from human and animal studies indicate that boron should be recognized as being of nutritional importance.

Key words: calcium; estrogen; brain function; trace elements; reactive oxygen; copper

INTRODUCTION

Although the consideration that boron is an essential nutrient for higher animals including humans is a relatively recent phenomenon, the first evidence that it may be an element of biological importance probably appeared in 1857 when the presence of boron was detected in some plant seeds [1]. It was not until 1910, however, that boron was suggested as being essential for higher plants [2]. Conclusive evidence and acceptance of the essentiality of boron for plants appeared in 1923 when Warington [3] reported that none of the 52 elements she tested could alleviate the signs of boron deprivation in several species of leguminous plants. Shortly thereafter, Sommer and Lipman [4] provided evidence that boron was essential for the completion of the life

*Correspondence to: Forrest H. Nielsen, United States Department of Agriculture, ARS, GFHNRC, P.O. Box 9034, University Station, Grand Forks, ND 58202-9034.

Received 28 September 1996; Accepted 28 October 1996

cycle of a number of plants. Interestingly, in the over 70 years since those reports, there have been extensive research efforts to elucidate the primary role of boron in the metabolism of vascular plants; yet today a specific biochemical role for boron in plants has not been conclusively identified. Nonetheless, there is universal agreement that boron is an essential nutrient for vascular plants.

About 15 years after the work of Warington [3], some eminent scientists in nutrition reported that they could not show that rats were adversely affected by diets containing only 155 to 163 ng B/g [5–7]. It was these reports that probably resulted in generations of students in nutrition, physiology, and biochemistry being taught that boron was essential for plants but not for animals.

In 1981, however, this dogma began to change. In that year, Hunt and Nielsen [8] reported that boron deprivation depressed the growth of chicks with the effect seemingly more marked when dietary cholecalciferol was deficient. Morphological examination of the tibias of the chicks also indicated that an interaction between boron and cholecalciferol affected bone formation. Rachitic long bones were found in 17 of 21 boron-deprived chicks, but only 9 were found in 22 boron-supplemented chicks, fed a cholecalciferol-deficient diet; moreover, the lack of calcification generally was more severe in the boron-deprived chicks.

Since 1981 numerous experiments with animals have shown that boron deprivation affects the function or composition of several body components including the skeleton and brain, and affects biochemical indices associated with the metabolism of several other nutrients including calcium, copper, nitrogen, and cholecalciferol. In many of the experiments the response to low dietary boron was enhanced when the animal model was simultaneously exposed to a stressor such as a diet deficient in calcium, cholecalciferol, magnesium, or potassium. Attention to the possibility that boron is an essential nutrient for humans was intensified by a study reported in 1987, which found boron deprivation increased the urinary excretion of calcium and decreased 17B-estradiol concentrations in postmenopausal women [9]. It is subsequent human experiments, however, that have produced the most convincing circumstantial evidence that boron is an essential element for humans; these findings will be accentuated here.

DESIGN OF THE HUMAN EXPERIMENTS

In the first of the subsequent experiments, designated as experiment 1 here and described in more detail elsewhere [10,11], the subjects were five men over the age of 45, four postmenopausal women, five postmenopausal women on estrogen therapy, and one premenopausal (or perimenopausal) woman (originally thought to be postmenopausal). After a 14-day equilibration period, the subjects were fed a boron-deficient diet, or about 0.25 mg/2,000 kcal, for 63 days and then fed the same diet supplemented with 3.0 mg B/day for 49 days. The diet was low in magnesium, about 115 mg/2,000 kcal, and marginally adequate in copper, about 1.6 mg/2,000 kcal, throughout the study.

In the second of the subsequent experiments, designated as experiment 2 here and described in more detail elsewhere [12,13], the subjects were four men over the age of 45, four postmenopausal women, five postmenopausal women on estrogen therapy, and, once again, one women who was thought to be postmenopausal but estrogen analyses during the study revealed that she was not. After a 14-day equilibration

period, there was a 63-day depletion period during which the basal diet containing about 0.25 mg B/2,000 kcal was fed; this was followed by a 49-day repletion period when the basal diet was supplemented with 3.0 mg B/day. During the first 32 days of this experiment, the diet provided about 1.7 mg Cu/2,000 kcal; this was lower than intended. Thus, from day 33 onward, the diet was supplemented to contain 2.4 mg Cu/2,000 kcal. Also, at an intake of 2,000 kcal, the diet provided about 300 mg of magnesium. Thus, the major differences between the two experiments were the intakes of copper and magnesium; in experiment 1 they were marginal or inadequate, and in experiment 2 they were adequate.

In both experiments, the subjects were fed a 3-day menu rotation diet that contained conventional foods including beef, pork, rice, bread, and milk but was low in fruits and vegetables. The energy of the diet was 11% as protein, 54% as carbohydrate, and 35% as fat. The 3 mg boron supplement was consumed at mealtimes in three divided doses as sodium borate in gelatin capsules.

Blood was drawn weekly by using standard phlebotomy techniques between 7:00 and 9:00 a.m. after 9 hours of fasting. Variables presented were determined by using commercially available kits or published methods [see 10–13 for details]. Although the subjects in both experiments were allowed a short equilibration period of 14 days, all variables examined did not seem to stabilize into a steady change or plateau at that time. Thus, to limit the influence of factors other than boron depletion, only the values obtained during the last 42 days of depletion in experiment 1, and last 35 days of depletion in experiment 2, were used in the statistical comparisons with the values obtained in the last 35 days of boron repletion. For each variable, a mean was computed for each dietary period for each subject. Paired t-tests of the means were then used to test for dietary effects [14]. In this test, each subject was his or her own control. A P value of 0.05 was considered significant.

FINDINGS INDICATING THE NUTRITIONAL ESSENTIALITY OF BORON

After being fed a boron-low diet for 63 days, a boron supplement of 3 mg/day affected the metabolism of a variety of substances in humans; many of these have been found to be similarly affected in experimental animals whose dietary boron was manipulated in an analogous manner. The dietary boron manipulations also apparently affected blood composition, brain function and psychomotor performance, the response to estrogen ingestion, and the response to marginal copper plus inadequate magnesium intake. A number of these effects are described in the following.

Calcium Metabolism

In experiment 1 when dietary copper and magnesium were marginal or inadequate, plasma ionized calcium, and serum 25-hydroxycholecalciferol and magnesium were lower, and serum calcitonin and osteocalcin were higher during the boron depletion period than the boron repletion period [11]. Only serum 25-hydroxycholecalciferol has been reported to be affected by the dietary boron manipulations in experiment 2 where dietary copper and magnesium were adequate [13].

Table I shows that in experiment 1 the difference in serum calcitonin with different dietary boron was significant when all subjects were combined (boron depletion was 74 pg/mL, and boron repletion was 59 pg/mL; $P<0.0008$), and similar changes oc-

TABLE I. Effect in Humans of Boron on Serum Calcitonin and 25-Hydroxycholecalciferol

Dietary boron[a] mg/day	Calcitonin pg/ml		25-OH-cholecalciferol ng/mL	
	Exp 1[b,c]	Exp 2[d]	Exp 1[b,c]	Exp 2[d,e]
Men over the age of 45				
0.25	71	36	25	18
3.25	60	34	29	26
P value	0.16	0.91	0.15	0.34
Postmenopausal women				
0.25	78	47	29	18
3.25	52	44	34	20
P value	0.02	0.37	0.23	0.75
Postmenopausal women on estrogen therapy				
0.25	61	33	36	18
3.25	55	28	37	30
P value	0.02	0.24	0.13	0.06
Above combined plus one premenopausal woman				
0.25	74	39	29	18
3.25	59	35	32	25
P value	0.0008	0.31	0.01	0.04

[a]After an equilibration period of 14 days when dietary boron was about 3.25 mg/day, there was a depletion period of 63 days when dietary boron was 0.25 mg/2,000 kcal followed by a repletion period of 49 days when the basal diet was supplemented with 3.0 mg boron/day as sodium borate.
[b]Dietary magnesium was inadequate (115 mg/2,000 kcal) and copper was marginal (1.6 mg/2,000 kcal) throughout the study.
[c]Data from Nielsen et al. [11].
[d]Dietary magnesium and copper were adequate, 300 mg and 2.4 mg/2,000 kcal, respectively.
[e]Data from Nielsen et al. [13].

curred when each group was analyzed separately; however, the difference with men did not achieve significance. In experiment 2, calcitonin values were lower than in experiment 1 and were not significantly affected by the dietary boron manipulations. These findings suggest that, because the calcitonin values in experiment 2 are close to those reported by others [15,16], the combined magnesium-low, copper-marginal diet in experiment 1 resulted in elevated serum calcitonin indicative of an abnormal calcium metabolism, and that boron deprivation exacerbated this abnormality. In experiment 2, because the magnesium and copper deprivation were not present to stress calcium metabolism and result in an elevated calcitonin, the dietary boron manipulations did not induce significant changes in serum calcitonin. Both elevated plasma calcitonin [15] and magnesium deprivation [17] have been associated with postmenopausal osteoporosis. Thus, the calcitonin changes induced by boron supplementation after boron depletion in experiment 1 can be construed as beneficial and indicative that boron nutriture can affect calcium metabolism in humans.

Further evidence that dietary boron can affect calcium metabolism is the 25-hydroxycholecalciferol findings shown in Table I. In both experiments, when all subjects were combined, the serum 25-hydroxycholecalciferol concentration was lower during boron depletion than boron repletion. Low serum 25-hydroxycholecalciferol is often considered detrimental to maintaining healthy bone. For example, serum 25-hydroxycholecalciferol concentrations were found to be lower in postmenopausal osteoporotic women than age-matched controls [18].

TABLE II. Effect in Humans of Boron on Serum Glucose, Triglycerides, and Creatinine

Dietary boron[a] mg/day	Glucose, mg/dL Exp 1[b,c]	Triglycerides, mg/dL Exp 2[d,e]	Creatinine Exp 1[b,c]	Creatinine Exp 2[d]
Men over the age of 45				
0.25	94	121	1.16	1.18
3.25	90	128	1.04	1.15
P value	0.21	0.55	0.0006	0.66
Postmenopausal women				
0.25	94	111	0.94	0.89
3.25	88	122	0.86	0.85
P value	0.007	0.01	0.25	0.02
Postmenopausal women on estrogen therapy				
0.25	91	143	0.98	0.99
3.25	86	175	0.89	0.96
P value	0.009	0.02	0.0008	0.07
Above combined plus one premenopausal woman				
0.25	93	125	1.03	1.01
3.25	88	142	0.93	0.98
P value	0.0004	0.004	0.0001	0.03

[a]After an equilibration period of 14 days when dietary boron was about 3.25 mg/day, there was a depletion period of 63 days when dietary boron was 0.25 mg/2,000 kcal followed by a repletion period of 49 days when the basal diet was supplemented with 3.0 mg boron/day as sodium borate.
[b]Dietary magnesium was inadequate (115 mg/2,000 kcal) and copper was marginal (1.6 mg/2,000 kcal) throughout the study.
[c]Data from Nielsen et al. [10].
[d]Dietary magnesium and copper were adequate, 300 mg and 2.4 mg/2,000 kcal, respectively.
[e]Data from Nielsen et al. [13].

Findings with animals also indicate that boron has an essential role that affects calcium metabolism, especially if it is stressed by other dietary manipulations such as a vitamin D deficiency. Animal findings similar to those described above for humans include, in boron-deprived, cholecalciferol-deficient chicks, boron supplementation increased plasma 1,25-dihydroxycholecalciferol [19], and serum 25-cholecalciferol and ionized calcium concentrations [20]. These effects were not seen in chicks fed adequate cholecalciferol. In vitamin D-deficient rats, low dietary boron decreased the apparent absorption and retention of calcium [21].

Energy Substrate Metabolism

Table II shows that dietary boron also affects energy substrates such as glucose and triglycerides in humans. In experiment 1, serum glucose concentrations were significantly higher during boron depletion than boron repletion when the comparison included all subjects. The difference also was significant in both postmenopausal women groups when they were analyzed separately. Unfortunately, serum glucose was not determined in experiment 2; however serum triglycerides were. As shown in Table II, in both postmenopausal women groups analyzed separately and with the comparison using all subjects combined, serum triglycerides were significantly lower during boron depletion than boron repletion.

Findings with animals also indicate that boron nutriture affects energy substrate

metabolism; findings similar to those found in the human studies include the following. In chicks, boron deprivation exacerbated the cholecalciferol deficiency-induced elevation in plasma glucose and decrease in serum triglycerides [20,22–24]. Boron deprivation also has been found to decrease serum triglyceride concentrations in rats [25].

Further study is required to clearly establish how boron affects energy substrate metabolism. Hunt [24] has suggested that boron has effects through regulatory-type inhibition of some enzymes involved in energy metabolism, through an effect on the metabolism of cholecalciferol which can affect energy substrate utilization, or through an effect on insulin action. Bakken and Hunt [19] found that peak insulin excretion was higher from pancreas isolated from boron-deprived than -supplemented chicks.

Nitrogen-Containing Metabolites Metabolism

In both experiments, blood urea nitrogen (BUN), serum creatinine, and urinary urea were significantly higher during boron depletion than boron repletion for all subjects combined, and, with a few exceptions, for each group separately [10,13]. Originally, these findings were thought to be an indication of a change in kidney function [10]; that is, the excretion or reabsorption of nitrogen metabolites was being changed by boron deprivation. However, the findings in Table III suggest another possibility. Instead of an expected decrease in urinary urea excretion with an increase in BUN during boron depletion, excretion was significantly increased when the comparison included all subjects. In experiment 2, urinary hydroxyproline excretion was determined; the excretion was significantly lower during boron depletion than repletion when all subjects were used in the comparison (Table III). Increased BUN, serum creatinine (Table II), and urinary urea, and decreased urinary hydroxyproline, suggest an alteration in amino acid or protein metabolism. In other words, the utilization of some amino acids or proteins are affected by boron such that the incorporation of amino acids into, or the breakdown of proteins, is changed and results in altered concentrations in nitrogen metabolites in blood and urine.

Some limited animal findings also suggest that dietary boron affects nitrogen metabolite metabolism. Plasma albumin and uric acid concentrations were found to be higher in boron-deprived than -supplemented rats [26] and magnesium-adequate chicks [24,27]; in magnesium-inadequate chicks, boron deprivation decreased plasma uric acid [24,27]. Boron deprivation was found to decrease plasma threonine and serine, and increase plasma glutamic acid in rats (Nielsen, F.H., unpublished). These findings suggest that the utilization of some amino acids, protein metabolism, or the elimination of nucleotides is affected by boron nutriture.

Reactive Oxygen Species Metabolism

Table IV shows that boron nutriture affects reactive oxygen species metabolism (ROS) in humans. Both superoxide dismutase and ceruloplasmin are enzymes involved in the protection against damage caused by ROS. In both experiments, erythrocyte superoxide dismutase (ESOD) was significantly lower during boron depletion than boron repletion if the comparison included all subjects or just the women on estrogen therapy [10,13]. Although the mean ESOD values were lower during boron depletion than boron repletion for the other group comparisons, significance was only achieved with the postmenopausal women not on estrogen therapy in experiment 2.

TABLE III. Effect in Humans of Boron on Blood Urea Nitrogen (BUN) and Urinary Urea and Hydroxyproline (OH-PRO)

Dietary boron[a] mg/day	BUN		Urinary urea g/day		Urinary OH-PRO mmol/day
	Exp 1[b,c]	Exp 2[d,e]	Exp 1[b]	Exp 2[d]	Exp 2[d]
Men over the age of 45					
0.25	14.6	14.6	9.22	8.15	0.0418
3.25	12.2	12.9	8.66	7.45	0.0470
P value	0.02	0.01	0.09	0.23	0.36
Postmenopausal women					
0.25	13.0	13.8	6.45	6.96	0.0546
3.25	11.4	12.6	6.40	6.61	0.0706
P value	0.07	0.08	0.88	0.47	0.06
Postmenopausal women on estrogen therapy					
0.25	13.1	13.4	6.69	6.78	0.0520
3.25	11.8	11.5	6.08	5.52	0.0609
P value	0.03	0.03	0.0008	0.02	0.05
Above combined plus one premenopausal woman					
0.25	13.5	13.8	7.48	7.07	0.0495
3.25	11.6	12.2	7.03	6.42	0.0601
P value	0.0001	0.0001	0.004	0.03	0.001

[a]After an equilibration period of 14 days when dietary boron was about 3.25 mg/day, there was a depletion period of 63 days when dietary boron was 0.25 mg/2,000 kcal followed by a repletion period of 49 days when the basal diet was supplemented with 3.0 mg boron/day as sodium borate.
[b]Dietary magnesium was inadequate (115 mg/2,000 kcal) and copper was marginal (1.6 mg/2,000 kcal) throughout the study.
[c]Data from Nielsen et al. [10].
[d]Dietary magnesium and copper were adequate, 300 mg and 2.4 mg/2,000 kcal, respectively.
[e]Data from Nielsen et al. [12].

The ceruloplasmin findings apparently were modified by dietary copper and magnesium. In experiment 1, when dietary magnesium was inadequate and copper was marginal, enzymatic ceruloplasmin was significantly lower during boron depletion than boron repletion when the comparison included all subjects. In experiment 2, when both dietary magnesium and copper were adequate, the dietary boron manipulations had no effect on enzymatic ceruloplasmin. On the other hand, immunoreactive (RID) ceruloplasmin was significantly lower during boron depletion than boron repletion when the comparison included all subjects, or just the postmenopausal women on estrogen therapy. The difference approached significance ($P<0.06$) when the comparison included just the men or postmenopausal women not on estrogen therapy.

Most likely, boron does not directly participate in the conversion of ROS into harmless metabolites but instead affects the formation of ROS during normal metabolism. This impression is supported by both plant and animal findings.

In plants, boron stimulates ascorbate free radical reduction to ascorbate by NADH oxidase [28]. Moreover, boron deficiency increases the antioxidant enzymes superoxide dismutase, catalase, and peroxidase in plants [29]; this is opposite of the human findings presented in Table IV. Perhaps this contrast occurred because the induction in the formation of ROS by changes in boron status has a different basis in humans and plants, and the antioxidant enzymes responded accordingly.

TABLE IV. Effect in Humans of Boron on Erythrocyte Superoxide Dismutase (SOD) and Serum Ceruloplasmin

Dietary boron[a] mg/day	SOD, U/g Hb		Ceruloplasmin, mg/dL		
			Enzymatic		RID
	Exp 1[b,c]	Exp 2[d,e]	Exp 1[b]	Exp 2[d]	Exp 2[d,e]
Men over the age of 45					
0.25	2,287	3,091	39.9	37.6	25
3.25	2,552	3,231	42.0	38.7	28
P value	0.41	0.79	0.46	0.53	0.06
Postmenopausal women					
0.25	2,213	2,666	49.6	46.9	30
3.25	2,386	3,169	53.8	47.9	33
P value	0.57	0.04	0.04	0.27	0.06
Postmenopausal women on estrogen therapy					
0.25	2,160	2,520	58.2	66.3	42
3.25	2,736	3,327	62.3	69.9	50
P value	0.04	0.03	0.35	0.53	0.05
Above combined plus one premenopausal woman					
0.25	2,257	2,735[f]	47.9	51.2	33
3.25	2,578	3,243[f]	51.2	53.1	38
P value	0.03	0.04	0.04	0.12	0.002

[a]After an equilibration period of 14 days when dietary boron was about 3.25 mg/day, there was a depletion period of 63 days when dietary boron was 0.25 mg/2,000 kcal followed by a repletion period of 49 days when the basal diet was supplemented with 3.0 mg boron/day as sodium borate.
[b]Dietary magnesium was inadequate (115 mg/2,000 kcal) and copper was marginal (1.6 mg/2,000 kcal) throughout the study.
[c]Data from Nielsen et al. [10].
[d]Dietary magnesium and copper were adequate, 300 mg and 2.4 mg/2,000 kcal, respectively.
[e]Data from Nielsen et al. [44].
[f]Values do not include the premenopausal woman.

Only circumstantial evidence has been reported on the possibility that boron affects ROS formation or destruction in experimental animals. As indicated above, boron nutriture modifies energy substrate metabolism; this metabolism yields ROS. Moreover, boron deprivation enhances histological abnormalities in the epiphyseal growth plate caused by cholecalciferol deprivation in the chick (24]. These abnormalities have been associated with changes in ROS.

Response to Estrogen Ingestion

Table V shows that estrogen ingestion elevated serum 17β-estradiol; in experiment 2, the elevation was significantly higher during boron repletion than boron depletion. In experiment 1, an apparently similar boron effect only approached significance ($P<.09$). Table V also shows that in both experiments estrogen elevated plasma copper; the elevation was significantly higher during boron repletion than boron depletion. Dietary boron did not affect plasma copper in the men or postmenopausal women not ingesting estrogen. The plasma copper findings did not mirror the serum ceruloplasmin findings shown in Table IV. Boron status did not affect the increase in both enzymatic and RID ceruloplasmin apparently induced by estrogen ingestion. The serum triglycerides findings in Table II have some aspects similar to the plasma

TABLE V. Effect in Humans of Boron on Serum 17β-Estradiol and Plasma Copper Concentrations*

Dietary boron[a] mg/day	17β-Estradiol, pg/mL		Copper, μg/dL	
	Exp 1[b]	Exp 2[c]	Exp 1[b]	Exp 2[c]
Men over the age of 45				
0.25	33	20	91	83
3.25	35	17	93	86
P value	0.50	0.12	0.71	0.14
Postmenopausal women				
0.25	23	11	127	107
3.25	18	11	128	108
P value	0.26	0.86	0.75	0.71
Postmenopausal women on estrogen therapy				
0.25	107	99	141	146
3.25	145	157	152	159
P value	0.09	0.02	0.03	0.04
Above combined				
0.25	52	48	117	115
3.25	64	69	122	121
P value	0.13	0.06	0.03	0.02

*Data from Nielsen et al. [8].

[a]After an equilibration period of 14 days when dietary boron was about 3.25 mg/day, there was a depletion period of 63 days when dietary boron was 0.25 mg/2,000 kcal followed by a repletion period of 49 days when the basal diet was supplemented with 3.0 mg boron/day as sodium borate.

[b]Dietary magnesium was inadequate (115 mg/2,000 kcal) and copper was marginal (1.6 mg/2,000 kcal) throughout the study.

[c]Dietary magnesium and copper were adequate, 300 mg and 2.4 mg/2,000 kcal, respectively.

copper findings and some similar to the serum ceruloplasmin findings. Estrogen apparently increased serum triglycerides; as with plasma copper, the increase was higher during boron repletion than boron depletion. However, as with serum ceruloplasmin, serum triglycerides of postmenopausal women not ingesting estrogen were also affected; they were higher during boron repletion than depletion. These findings indicate that boron can both enhance and mimic the effects of estrogen ingestion.

Copper Metabolism

Several of the preceding findings indicate that there is a relationship between boron and copper. Not only was the response to the dietary boron manipulations apparently affected by dietary copper, several indices of copper metabolism were affected. These include plasma copper, serum ceruloplasmin, and ESOD. Moreover, boron nutriture affected several variables that have been shown to be affected by dietary copper, including 25-hydroxycholecalciferol [30], glucose [31], and triglycerides [32]. Boron nutriture also affects copper metabolism in animals. For example, boron deprivation decreased the copper concentration in bone of chicks [33].

Blood Composition

Table VI shows that when dietary copper and magnesium were adequate (experiment 2), the boron manipulations affected the cell composition of blood and the hemoglobin concentration in the red blood cell (RBC). The amounts of RBCs and

TABLE VI. Effect of Boron on Red Blood Cell Number (RBC), White Blood Cell Number (WBC), Platelet Number, Mean Corpuscular Hemoglobin Concentration (MCHC), and Serum Iron and Ferritin in Humans Fed Adequate Copper and Magnesium (Exp 2)*

Dietary boron[a] mg/day	RBC 10^{12}/L	WBC 10^9/L	Platelets 10^9/L	MCHC %	Serum iron μg/dL	Serum ferritin μg/L
Men over age 45						
0.25	4.83	5.14	280	33.9	80	19.1[b]
3.25	4.64	5.20	256	34.8	82	14.0
P value	0.007	0.45	0.008	0.002	0.78	0.35
Postmenopausal women						
0.25	4.35	5.39	312	33.7	85	34.5
3.25	4.28	5.44	278	34.7	72	25.0
P value	0.30	0.81	0.007	0.03	0.08	0.16
Postmenopausal women on estrogen therapy						
0.25	4.30	6.10	297	33.6	85	21.1
3.25	4.19	6.33	268	35.4	84	16.0
P value	0.03	0.53	0.007	0.005	0.90	0.17
Above combined plus one premenopausal woman						
0.25	4.46	5.54	297	33.7	83	22.9
3.25	4.34	5.74	269	35.0	79	17.8
P value	0.0002	0.19	0.0001	0.0001	0.19	0.04

*Data from Nielsen et al. [12].
[a]After an equilibration period of 14 days when dietary boron was about 3.25 mg/day, there was a depletion period of 63 days when dietary boron was 0.25 mg/2,000 kcal followed by a repletion period of 49 days when the basal diet was supplemented with 3.0 mg boron/day as sodium borate.
[b]Comparisons made with data in ln transformed form.

platelets in blood were significantly lower during boron repletion than boron depletion when the comparison included all subjects combined or each group separately. Although the mean count of white blood cells (WBC) was slightly higher during boron repletion than boron depletion, no comparison was significant. However, in a recent study involving 43 perimenopausal women, a 3.0 mg/day boron supplement significantly increased WBC counts (Nielsen, F.H., unpublished). Although RBC counts were diminished during the boron repletion period, the mean corpuscular hemoglobin concentration (MCHC) was not; in fact, it was significantly higher during boron repletion than depletion with every comparison. The ferritin and iron data in Table VI show that phlebotomy and the dietary boron manipulations apparently did not markedly affect iron status. Thus, the effects on blood cell numbers and MCHC can not be attributed only to a change in iron status.

Reported findings showing that dietary boron affects blood composition in animals are scarce. There have been reports that boron deprivation decreases hematocrits and hemoglobin concentrations slightly but significantly in rats [34] and chicks [33] under some situations.

The magnitude of the effect of boron on RBC counts and hemoglobin suggests that the changes are not of clinical significance. Most likely the boron effect is indirect, but the changes may provide clues as to the possible physiological role of boron. Both the cellular internalization of iron needed for hemoglobin formation and the action of erythropoietin (the primary humoral agent that regulates erythropoiesis) involve the binding of transferrin and erythropoietin, respectively, to cell membranes. Perhaps

TABLE VII. Effect in Humans of Boron on Selected Cognitive and Psychomotor Performance Variables*

	Experiment 1[a] Dietary boron,[c] mg/day		Experiment 2[b] Dietary boron,[c] mg/day	
Test	0.25	3.25	0.25	3.25
Tapping (number tapped/30 sec)				
All sequences	22.1[d]	24.2	23.2[e]	24.6
2-key sequences	30.2[d]	32.5	32.3[e]	33.6
4-key sequences	14.0[d]	15.9	14.1[d]	15.5
Search only response times (s)	3.43[d]	2.63	3.52[d]	3.02
Search and count response times (s)				
All conditions	7.05[d]	6.27	7.73[d]	6.84
Targets present	6.95[d]	6.18	7.53[d]	6.72
Targets absent	8.11[e]	6.83	8.66	8.15
Symbol-digit				
Response times (s)	2.14[d]	1.88	2.27[d]	1.98
Error	3.25	2.85	2.78	2.26

*Data from Penland [35].

[a]Dietary magnesium was inadequate (115 mg/2,000 kcal) and copper was marginal (1.6 mg/2,000 kcal) throughout the study.

[b]Dietary magnesium and copper were adequate, 300 mg and 2.4 mg/2,000 kcal, respectively.

[c]After an equilibration period of 14 days when dietary boron was about 3.25 mg/day, there was a depletion period of 63 days when dietary boron was 0.25 mg/2,000 kcal followed by a repletion period of 49 days when the basal diet was supplemented with 3.0 mg boron/day as sodium borate.

[d]$P < 0.05$.

[e]$P < 0.01$.

boron affects RBC hemoglobin and blood cell formation or breakdown through an effect at the cell membrane level. As describe vide infra, it has been hypothesized that boron has a biological role that affects cell membrane function or stability.

Brain Function and Cognitive and Psychomotor Performance

Penland [35] has reported that a low boron intake results in electroencephalogram (EEG) changes suggestive of reduced behavioral activation (e.g., drowsiness) and mental alertness. In addition, Penland [35] found that the EEG changes seem to be in concert to the finding that a low boron intake results in poorer performance in tasks that involve psychomotor skills and the cognitive processes of attention and memory. As shown in Table VII, with the psychomotor task of tapping, when contrasted with an adequate boron intake, low dietary boron resulted in fewer complete sequences tapped overall and fewer taps for both long and short sequences in both experiments. With the attention task of search-count, low dietary boron increased response times during search-and-count and search-only in both experiments. Finally, with the memory task of symbol-digit recognition, low dietary boron increased response times to encode and recall symbol-digit pairings in both experiments.

Brain function and composition of rats are also affected by dietary boron. Penland [36] found that boron deprivation systemically influenced brain electrical activity assessed by electrocorticograms in mature rats; the principal effect was on the frequency distribution of electrical activity. In this study, brain copper concentrations

were higher in boron-deprived than in boron-supplemented rats. Calcium concentrations in total brain and in brain cortex, as well as phosphorus concentration in cerebellum, have also been found to be higher in boron-deprived than in boron-supplemented rats fed a vitamin D-deficient diet [21].

The findings show that relatively short periods of restricted boron intake can affect brain function and cognitive performance in otherwise healthy adults. Penland [35] has stated that the changes induced by low dietary boron are similar, but not as severe, as those found with malnutrition or some metal toxicities.

DISCUSSION

The findings above show that boron is a dynamic trace element which, in physiological amounts, can affect the metabolism or utilization of numerous substances involved in life processes including calcium, copper, magnesium, nitrogen, glucose, triglyceride, reactive oxygen, and estrogen. Through these effects, boron can affect the function or composition of several body systems including blood, brain, and skeleton, generally in a beneficial fashion. However, boron is not generally recognized as essential or nutritionally important for humans; this is probably because a specific biochemical function for boron has not been elucidated or, as demonstrated for plants, it has not been shown necessary to complete the life cycle. Nonetheless, because the effects of boron in animals and humans are similar in many ways to those in plants, a reasonable conclusion is that boron is an essential nutrient for most all living things.

The identification of the biochemical role of boron is urgently needed so that dietary guidance can be given for boron. At least 15 hypotheses have been advanced as to the specific essential function of boron in plants [37,38], in which boron deficiency has a multiplicity of effects as it does in animals and humans. These hypotheses have boron involved in sugar transport, cell wall synthesis and lignification, cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid metabolism, phenol metabolism, membrane function, and DNA synthesis. The hypotheses receiving the most attention at present are those that have boron with a function with a cascade effect; that is, in cell wall or in membrane function. The evidence that boron is involved in lignin biosynthesis and cell wall cross-linking, including the finding that plants grown on sufficient boron media bend easily while plants grown on low boron media are brittle, has been reviewed [37]. The hypothesis that is of interest here, because the role may be similar to that in the animal kingdom, is that of boron having a regulatory role involving plant hormones and the control of a second messenger such as calcium at the cell membrane level [39]. This hypothesis is supported by findings that boron influences membrane potential and proton movement through membranes of plant cells [40]. Suggestions for the nature of the role of boron in plant membranes include an influence on cell lipid biosynthesis [41], the bridging of lipids via hydroxy groups [41], and the formation of complexes with messenger molecules containing inositol [40]. Neither boron-lipid nor boron-inositol complexes have been isolated from plant membranes.

Another hypothesis is that boron is involved in ascorbate metabolism in the plant plasmalemma [41]. Plasmalemma NADH oxidase is stimulated by boron [28]. The role of NADH oxidase in plant metabolism is unknown but it has been speculated to

be involved in the reduction of ascorbate-free radical to ascorbate [41]. Through an effect on ascorbate metabolism, boron possibly affects cell wall formation by influencing proline hydroxylation, or membrane transport through influencing redox reactions. Evidence that boron affects ascorbate metabolism includes the finding that ascorbate supplementation restores growth of the squash root meristem retarded by boron deprivation [41].

Two hypotheses recently have been advanced for the biochemical function of boron in higher animals that accommodate the large and varied response to boron deprivation. Hunt [24] has proposed that boron is a metabolic regulator through complexing with a variety of substrate or reactant compounds in which there are hydroxyl groups in favorable positions (cis-diols). Based upon the knowledge that two classes of enzymes are competitively inhibited in vitro by borate or its derivatives, and upon his findings that show boron can alter in vivo activity of a number of these enzymes, Hunt has hypothesized that the metabolic regulation by boron is mainly negative; that is, boron controls a number of metabolic pathways by competitively inhibiting some key enzyme reactions. Several years ago, I hypothesized that boron has a role in cell membrane function or stability such that it influences the response to hormone action, transmembrane signalling or transmembrane movement of regulatory cations or anions [42]. This hypothesis is supported by the recent finding that boron influences the transport of extracellular calcium into rat platelets activated by thrombin [43,44]. The hypothesis is based upon the concept that boron reacts with hydroxyl groups of phosphoinositides and glycolipids of membranes. It also could be based upon the hypothesis put forward for plants; that is, boron influences redox reactions involved membrane transport.

CONCLUSION

Regardless of the uncertainty about the specific biochemical mechanisms through which it acts, there is overwhelming circumstantial evidence indicating that boron is of nutritional importance. If boron is not essential in the classical sense, it certainly could be considered beneficial in humans exposed to certain nutritional stressors such as vitamin D, copper, and magnesium deficiency. Thus it seems appropriate to recommend that people eat a diet that provides luxuriant amounts of boron. Findings from the human studies described indicate that subjects consuming about 0.25 mg B/day responded positively to boron supplementation of 3.0 mg/day. This suggests that boron intakes should be higher than 0.25 mg/day. Extrapolations from animal data have resulted in the suggestion that humans may have a boron requirement between 0.5 and 1.0 mg/day [45]. Because many people consistently consume less than this amount by choosing diets low in fruits, vegetables, nuts, and legumes, boron most likely is of more clinical and nutritional importance than currently acknowledged.

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